PHYLOGENETICS OF HYACINTHACEAE BASED ON PLASTID DNA SEQUENCES[1]

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Abstract

Hyacinthaceae presently consist of approximately 70 genera and 1000 species. To investigate the monophyly of the family and the generic relationships, we sequenced the *trn*L intron and the *trn*L-*trn*F intergenic spacer region of chloroplast DNA for 105 taxa in Hyacinthaceae and 18 species of related families. By testing different outgroup compositions, we provide evidence for the monophyly of the family if the North American genera *Camassia* and *Chlorogalum*, which are more closely related to *Agave* and *Hosta*, are excluded from Hyacinthaceae sensu Dahlgren. Several generic implications can be deduced from the analysis, the most prominent one of which is the polyphyletic origin of the Linnaean genera *Scilla*, *Ornithogalum*, and *Hyacinthus*. Especially members of the genera *Scilla* and *Hyacinthus* are extensively intermixed with each other. According to the DNA sequence data, the only true *Scilla* species are found in the Mediterranean region and appear as a monophyletic clade. A tetrapartition of the family into (1) the monotypic subfamily Oziroëoideae Speta, accommodating the South American Hyacinthaceae; (2) the subfamily Urgineoideae Speta, housing relatives of the squills; (3) the subfamily Ornithogaloideae Speta, including the tribes Ornithogaleae Rouy and Dipcadieae Rouy; and (4) the largest and most advanced subfamily, Hyacinthoideae Link, consisting of the tribe Massonieae Baker (including species from Africa south of the Sahara and from India) and the Mediterranean/Asian tribe Hyacintheae Dumort., is proposed. Previously included in Hyacinthaceae sensu Dahlgren, the North American genera *Chlorogalum* and *Camassia* show affinities to Agavaceae and Funkiaceae and appear as a distinct clade together with Anthericaceae. Furthermore, the occurrence of taxa from southern Africa at basal positions in all subfamilies points to the origin of evolution of the Hyacinthaceae in this region.

For many systematists the bulbous plants represent the very center of Liliaceae (Cronquist, 1981). Therefore, it was a big surprise when Dahlgren et al. (1982, 1985) emphasized that these bulbous plants are members of two families, the Liliaceae sensu stricto and the Hyacinthaceae, which moreover belong to the different orders Liliales and Asparagales, respectively. However, the process of arriving at this systematic decision was not straightforward: The first circumscription of an independent plant family Hyacinthaceae by Batsch (1786) was not widely utilized by the scientific community. Batsch combined in this family rather heterogeneous liliaceous plants possessing flowers with a fused perianth. Among the 17 genera included within Hyacinthaceae sensu Batsch, only two (*Hyacinthus*, *Lachenalia*) are considered as belonging to the present circumscription of this plant family (Speta, 1998a, b). The genera *Ornithogalum*, *Scilla*, and *Albuca* Batsch placed within Alliaceae. In 1836 familial status was reduced to tribal level by Endlicher, who included species irrespective of the degree of perianth fusion. Interestingly, he also included the American genus *Camassia* in Hyacintheae. In his 1866 posthumously published fragment "The genera of plants," Salisbury re-evaluated the Linnaean genera *Hyacinthus*, *Scilla*, and *Ornithogalum* and redistributed often new genera into the families Eucomaceae, Lachenaliaceae, Hyacinthaceae, and Ornithogalaceae. Adopting a broader circumscription of Liliaceae, Baker, who obviously knew Salisbury's publication, refused to follow his ideas although he used tribes for his Liliaceae sensu lato. Hyacintheae and Scilleae were created in 1870, followed by Massonieae (1871) and Chlorogaleae (1873). Genera with floral fusion were placed in Hyacintheae, whereas those with more or less free tepals were accommodated within Scilleae. Later, Engler (1887) would separate bulbous Liliaceae, for him central to the family, into tribes Lilieae and Scilleae but considered these groups to be closely related. *Bowiea* and *Schizobasis* were included within Eriosperminae (Asphodeloideae–Asphodeleae) by Engler, whereas *Schoenolirion*, *Chlorogalum*, and *Hemiphylacus* were coalesced within Chlorogalinae with *Camassia* directly related to *Scilla* (Engler, 1887). His student Schulze (1893) simi-

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larly concluded that distinctions between Tulipeae and Scilleae were not morphologically warranted. It was early this century when Fritsch in Graz, together with his Ph.D. students, investigated Liliaceae sensu lato. Not only did their comparative anatomical studies substantiate the distinction of the Lilioideae into Tulipeae and Scilleae (Fuchsig, 1911), but they also arrived at the conclusion that *Bowiea* is rather isolated within Asphodeleae (Bouvier, 1915). Based on these results, Fritsch (1932) proposed the recognition of several smaller and more homogeneous families rather than keeping the conservative concept of a large and heterogeneous family Liliaceae.

A more profound contribution to the classification of Liliaceae was provided by Schnarf and students in Vienna (Schnarf, 1929; Wunderlich, 1937; Schmid, 1938; Buchner, 1949), utilizing comparative embryology. Building from them, Krause (1930) elevated Scilleae and Lilieae tribes to subfamilial status. Wunderlich (1937) moreover questioned, based on embryological characters, the retention of obviously different groups like Lilioideae and Scilloideae in the same family, with Scilloideae further shown as embryologically heterogenous. Two groups were distinguished: (1) an *Ornithogalum*-group consisting of genera *Ornithogalum*, *Muscari*, and *Puschkinia* with helobial endosperm and (2) a second group (*Scilla*, *Hyacinthus*, *Camassia*, *Galtonia*) with nuclear endosperm and more variation in embryo sac development. Further, Schmid (1938) divided Scilloideae on differences in pistil anatomy and pollen-tube leading tissue: (1) *Albuca*, *Galtonia*; (2) *Scilla*, *Camassia*, *Ornithogalum*, *Hyacinthus*, *Puschkinia*, *Muscari*; (3) *Ledebouria* (*Drimia* in her thesis), *Eucomis*, and *Veltheimia*. Later, Buchner (1949) supported Schmid's second group embryologically, grouping *Ornithogalum*, *Muscari*, and *Puschkinia*, then *Scilla* and *Hyacinthus* together. The status of *Camassia*, *Drimiopsis*, and *Urginea* remained unresolved.

In contrast to other families, Hyacinthaceae proved to be karyologically variable (as reviewed in Speta, 1998b). Nevertheless, chromosome numbers are usually constant at the genus level in Hyacinthaceae, irrespective of the genus size (*Scilla* sensu stricto: $x = 9$; *Muscari*: $x = 9$; *Bellevalia*: $x = 4$; *Albuca*: $x = 9$). However, the occurrence of dysploid series again complicated the situation (*Prospero*: $x = 4, 5, 6, 7$; *Barnardia*: $x = 8, 9$; *Hyacinthella*: $x = 9, 10, 11, 12$; *Stellarioides*: $x = 2, 3, 4, 5, 6, 7, 8, 9$; *Schnarfia*: $x = 9, 10$). Nevertheless, Huber (1969) accepted Wunderlich's (1937) proposal, independently recognizing Scilloideae as family Hyacinthaceae. Species were divided according to seed characters into tribes Chlorogaleae (*Chlorogalum*, *Schoenolirion*, and probably *Hemiphylacus*), Bowieae (*Bowiea*, *Schizobasis*), and Scilleae. For tribe Scilleae, Huber (1969) noted *Camassia* and *Ornithogalum* to be the basal genera. *Camassia* was the most basal genus of the blue-flowering group and was closely related to *Endymion* Dumort. [= *Hyacinthoides* Medicus] and *Scilla*. *Puschkinia* and *Chionodoxa* were closely related to *Scilla*. Another blue-flowering group includes *Hyacinthus* and *Muscari* sensu lato with *Lachenalia* and *Massonia* closely related to *Hyacinthus*. In contrast to blue-flowering Scilleae, which all show affinities to each other, Scilleae with non-blue flowers isolate into several groups. Some showed distant relationship to blue-flowering lines like *Lachenalia* and *Massonia*. For other genera (*Drimiopsis*, *Eucomis*, and *Veltheimia*) a more likely relationship to *Ornithogalum* was proposed. Close relationship was drawn between *Drimia* and *Galtonia*, *Dipcadi* and *Pseudogaltonia*, *Albuca* and *Urginea* (Huber, 1969).

Pollen features can be systematically valuable but not for Hyacinthaceae. Therefore, Schulze (1980) was reluctant to recognize the family status of Hyacinthaceae based on pollen characters. He only recognized the tribes Scilleae and Massonieae sensu Huber but did not include Chlorogaleae and Bowieae in Hyacinthaceae. Tribes Chlorogaleae and Bowieae were later included within Anthericaceae (Schulze, 1982).

As reviewed here, traditional methodologies have not resolved subfamilial relationships within Hyacinthaceae. Certain characters like perianth features are clearly non-informative. For example, *Chionodoxa* with its fused tepals was shown to be closely related with generitype *Scilla bifolia* L., which has more or less free tepals. *Chionodoxa* was therefore synonymized under *Scilla* (Speta, 1971, 1976).

Splitting of the large Linnaean genus *Hyacinthus* into a series of more homogeneous genera is now widely accepted. Conversely, the distribution of species of the Linnaean genus *Scilla* into distinct genera has not achieved a broad consensus. The broad circumscription of the genus *Scilla* by Linnaeus (1753), a name that was previously exclusively reserved for the squills, apparently had an adverse effect on the necessary dissection of this genus into more natural genera. Consequently, a new name had to be found for the relationship of the squills. In fact, a whole squill subfamily (Urgineoideae) is encapsulated within an obviously heterogeneous *Squilla* and an equally heterogeneous genus *Drimia* (Jessop, 1977; Stearn, 1978; Stedje, 1987). Only Jessop (1970) acknowledged an independent genus *Ledebouria* within African/In-

dian *Scilla* sensu lato. This was later substantiated by molecular data (Stedje, 1998), whereas other *Scilla* sensu lato relationships remained untouched.

The Hyacinthaceae have been long recalcitrant to a natural classification into subfamilies and tribes. One reason is the classification of most species into three highly heterogeneous genera, *Scilla*, *Hyacinthus*, and *Ornithogalum*, by Linnaeus. Efforts to find characters to discriminate at higher hierarchical levels within Hyacinthaceae have revealed the obsoleteness of most characters, which otherwise have been shown to be useful for classification in other families. Characters useful at higher taxonomic levels in other families often reveal a high variability among closely related species within Hyacinthaceae. For example, the type of the embryo sac or endosperm can be variable even within closely related species of *Scilla* sensu stricto (Svoma, 1981) or *Prospero* (Ebert, 1993). Likewise, chromosome numbers can be constant within certain genera but highly variable in other genera, resulting in aneuploid series difficult to interpret. Pollen grains of many genera in Hyacinthaceae do not show significantly different morphologies and are only of limited taxonomic value (Schulze, 1980). Chromosome counts and morphology have been phylogenetically useful at the species level but higher-order taxa remain problematic (Speta, 1979). Improvement of cytological techniques (chromosome banding, nuclear DNA amounts, etc.) has aided in the advancement of classification at species level but contributes little to higher-level systematics.

Chemotaxonomic features have been shown to be useful to distinguish genera within Hyacinthaceae and to group them at higher hierarchical levels. Cardiac-active steroids occur in several genera. One group, the bufadienolids, have been found only in species clearly related to *Charybdis maritima* (L.) Speta (Krenn, 1990, 1994) and thus delineate the subfamily Urgineoideae. A second group of cardiac-active compounds, cardenolids, have been found in several allies to *Ornithogalum* (subfamily Ornithogaloideae). However, investigation of species within Ornithogaloideae is not yet completed, and several members lack these compounds. It cannot be excluded therefore, that this subfamily may still contain additional characteristic compounds. *Zahariadia saundersiae* (Baker) Speta and *Eliokarmos thyrsoides* (Jacq.) Raf. are characterized by cholestan steroids (Kubo et al., 1992a, b). Furthermore, most species in *Ornithogalum* and allies exhibit protein crystals in their nuclei, a feature absent from other Hyacinthaceae (Speta, unpublished). An unrelated group of phenolic compounds (homoisoflavanoles) (Heller & Tamm, 1981) discriminate a third group of genera (subfamily Hyacinthoideae) from *Urginea*- and *Ornithogalum*-affined taxa. Interesting in this respect are serological investigations placing North American *Camassia* near Agavaceae (Cupov & Kutjavina, 1981), although they alternatively could be included in Hyacinthaceae due to chemotaxonomic and karyological features. An independent family Camassiaceae, sister to Agavaceae and Hesperocallidaceae, has even been proposed (Cupov, 1994). When Fay and Chase (1996) circumscribed the new family Themidaceae, they also sequenced the *rbc*L locus of a few genera in Hyacinthaceae. Again, *Camassia* and *Chlorogalum* showed affinities to Agavaceae.

At present the Hyacinthaceae accommodate approximately 1000 species distributed into roughly 70 genera (Speta, 1998a, 1998b). To evaluate generic relationships within the family Hyacinthaceae, we sequenced the *trn*L-intron and the intergenic spacer (IGS) between the *trn*L-(UAA)-3′-exon and *trn*F-(GAA) chloroplast gene, two DNA regions where sequence divergence is useful to resolve generic and subgeneric relationships (Fangan et al., 1994; Stedje, 1998). In total, we sequenced 123 accessions comprising 105 species and 51 genera of Hyacinthaceae, as well as four accessions of North American *Camassia* and *Chlorogalum* species, and 14 selected outgroup species from the lilioid families Agavaceae, Amaryllidaceae, Anthericaceae, Blandfordiaceae, Doryanthaceae, Funkiaceae, Hemerocallidaceae, Lomandraceae, and Nolinaceae. Furthermore, we included DNA sequence data from 12 published species of Hyacinthaceae, as well as unpublished sequences of 11 outgroup species, in our analysis. We interpret our results with reference to well-established morphological and cytogenetic data. From this phylogenetic perspective, we discuss the generic limitations within Hyacinthaceae as well as the monophyly of most genera as recognized by Speta (1998a, b).

Materials and Methods

Plant Material and DNA Extraction

Genetic accessions and herbarium vouchers of all plant materials are listed in Appendix 1. Nomenclature of taxa included in the study follows that of Speta (1998a, b).

DNA was extracted from 5–50 mg of plant material obtained either from fresh leaves or bulb scales or from herbarium specimens (*Whiteheadia etesionamibensis* Müller-Doblies, *Zagrosia persica* (Hausskn.) Speta acc. 1, *Scilla* cf. *bulgarica* Speta,

and *Urginavia micrantha* (A. Rich.) Speta) following the single tube isolation protocol of Steiner et al. (1995) with minor modifications. Lyophilized and powdered material was extracted with 400 μl ROSE buffer (10 mM Tris-HCl pH 8.0; 312.5 mM EDTA, pH 8.0; 1% sodium lauryl sarkosyl; and 1% polyvinylpolypyrrolidone) at 90°C for 10 minutes. After centrifugation, 10 μl of the supernatant were diluted 100-fold with TE buffer pH 8.0 and stored at 4°C. Two μl of the diluted DNA were used as a template in a 50 μl PCR reaction.

DNA AMPLIFICATION

Two noncoding regions of the chloroplast genome were sequenced. The *trn*L(UAA)-intron and the intergenic spacer (IGS) between *trn*L-(UAA)-3′-intron and *trn*F-(GAA) gene were amplified together in a single PCR reaction using the C- and F-primers as published by Fangan et al. (1994). The thermal cycling protocol comprised 30 cycles of 10 sec at 94°C, 10 sec at 45°C, and 20 sec at 72°C in a TouchDown thermal cycler (HYBAID), operated in tube-control mode. Amplified, double-stranded DNA fragments were purified using the Wizard DNA purification system from Promega.

DNA SEQUENCING

Purified PCR products were directly sequenced on an A.L.F. automated sequencer (Pharmacia) using 5′-fluorescein-labeled nested primers (two primers 5′-CTACGGACTTAATTGGATTGAGC-3′ and 5′-GGGGATAGAGGGACTTGAAC-3′ for the *trn*L-intron and two primers 5′-GGTTCAAGTCCCTCTATCCC-3′ and 5′-AGGATTTTCAGTCCTCTGCTC-3′ for the intergenic spacer) following the cycle sequencing protocol of the manufacturer (AutoCycle sequencing kit, Pharmacia). Both strands were sequenced, and in cases where ambiguities could not be resolved by comparison with the sequence of the complementary strand, additional PCR reactions were performed to generate new templates for sequencing. Ambiguities resulting from compressions were resolved by using the Thermo Sequenase fluorescent labeled primer cycle sequencing kit with 7-deaza-dGTP (Amersham).

DATA ANALYSIS

Sequence manipulations and phylogenetic analyses were performed on a Digital Alpha 1000A 5/400 server under the operating system Digital Unix V.4.0D. DNA sequences were prealigned by using the PileUp program of the GCG software Package (Genetics Computer Group, 1994). Final alignment of DNA sequences was done by eye. *Aspidistra elatior* was designated as the outgroup in phylogenetic reconstructions using test version 4.0d64 of PAUP* written by David L. Swofford. Using the gapcode = missing parameter the data matrix was subjected to 1000 replicates of random sequence additions using tree bisection-reconnection (TBR) branch-swapping under the Fitch criterion (unordered states and equal weights: Fitch, 1971). At each step only ten trees were permitted to be held to minimize the time the algorithms spent for searching for trees on sub-optimal islands.

Successive weighting was applied and new heuristic searches were performed using the trees of the previous analysis as starting trees until tree lengths remained the same in two successive rounds. Finally, 10,000 fast bootstrap (Felsenstein, 1985) replicates, as well as 10,000 replicates using the jackknife algorithm, were performed to assess confidence limits for tree topology using the final weight set from successive weighting. During all analyses the maximum number of trees in memory was limited to 10,000. Indels in the data matrix were coded as additional characters, and tree searches were performed using the nucleotide data alone or together with the indel data. Tree manipulations were performed using MacClade version 3.06 (Maddison & Maddison, 1992).

The DNA sequence data consisting of the *trn*L intron and the *trn*L-*trn*F intergenic spacer have been deposited in the EMBL data library (accession numbers, see Appendix 1). An aligned DNA matrix is available via electronic mail from MP upon request (email: martin@gem.univie.ac.at).

RESULTS

SEQUENCE VARIATION OF *TRNL*-INTRON AND *TRNL-TRNF* IGS REGIONS

In total, the combined and aligned intron+IGS sequences yielded 1297 characters with 452 parsimony-informative positions. Thirty indels, which mainly consisted of simple 4–5-bp-long sequence repeats and thus were easy to align, were coded and added to the data matrix. The lengths of the intron sequences varied between 515 and 592 nucleotides except for *Eliokarmos graminifolius* (Thunb.) Speta, which shows a large deletion in this region and therefore only contains 273 nucleotides, as well as the *Anthericum* and *Leucocrinum* sequences, which are likewise shorter (472–480 nt). The lengths of the IGS sequences ranged from 342 to 408 nucleotides with the exception of *Eustrephus latifolius* (265 nt), *Thysanotus virgatus* Brittan (324 nt), *Leucocrinum montanum* (267 nt), *Anthericum*

(296–299 nt), *Chlorogalum* (306 nt), *Albuca nelsonii* N. E. Br. (247 nt), *Whiteheadia etesionamibensis* Müller-Doblies (268 nt), *Hyacinthella* (271 nt), and *Pfosseria bithynica* (Boiss.) Speta (282 nt), which again exhibit deletions in this region. From nucleotide position 433 to 480 within the intron, a hypervariable AT-rich region occurred, evolving presumably from a di-nucleotide microsatellite. A similar hypervariable poly-T microsatellite was also found in the IGS region from nucleotide position 827 to 835. Since in both cases it was difficult to find unequivocal alignments, and different alignments in these regions always resulted in high homoplasy indices, both regions have been excluded from phylogenetic analyses.

PHYLOGENETIC ANALYSIS

Cladistic analysis of the combined nucleotide plus indel data set yielded more than 10,000 equally parsimonious trees of 1415 steps with a CI of 0.597 and RI of 0.862. Examination of approximately 50 randomly chosen trees revealed only minor differences in the placement of closely related taxa among different trees. One of the most parsimonious trees calculated from the combined nucleotide-indel data set is presented in Figures 1 and 2. Percent support from bootstrap analysis is shown above each branch. Statistical support for branching information using the jackknife procedure is indicated graphically by marking strongly supported clades (node score > 0.63) with closed circles and moderately supported clades (node score < 0.63 but > 0.5) with open circles. Phylogenetic analysis of the same data set but excluding indel data resulted in similar tree topology with only minor changes in bootstrap values (data not shown). Trees constructed using distance matrix analysis using neighbor joining did not deviate significantly from parsimony analysis but showed the tendency to misplace taxa with larger deletions in their sequences (data not shown).

RELATIONSHIPS

The ingroup relationships of all species representing Hyacinthaceae analyzed in this study are depicted in Figure 1, whereas the second part of the tree presenting the outgroup relationships is shown in Figure 2. There is a strong bootstrap support (100%) for the monophyly of the family, if the North American genera *Camassia* and *Chlorogalum* are excluded from Hyacinthaceae, but the monotypic South American genus *Oziroë* Raf. is included. Monophyly of most genera is also supported by bootstrap values: *Fessia* (76%), *Prospero* (87%), *Hyacinthella* (94%), *Scilla* (99%), *Muscari* (97%), *Bellevalia* (100%), *Hyacinthoides* (99%), *Tractema* (96%), *Oncostema* (100%), *Autonoë* (100%), *Drimiopsis* (84%), *Ornithogalum* (53%), *Albuca* (72%), *Dipcadi* (98%), *Charybdis* (<50%), *Rhadamanthus* (92%), *Oziroë* (100%). No single most recent ancestor for all analyzed species of the genera *Ledebouria, Lachenalia, Merwilla, Eliokarmos, Galtonia, Stellarioides*, and *Urginavia* can be found. The remaining genera are represented by a single species only, and therefore no information about the generic delineations can be deduced from the sequence data.

The classification of the Hyacinthaceae into the subfamilies Hyacinthoideae, Ornithogaloideae, Urgineoideae, and Oziroëoideae based on chemotaxonomic characters according to Speta (1998a, b) is also reflected in the cladogram. The monotypic South American subfamily Oziroëoideae Speta is the basal group (100%), followed by the Urgineoideae Speta (100%), a group of species that contains bufadienolids, the Ornithogaloideae Speta (100%), which are characterized by cardenolids and/or the presence of protein crystals in the nucleus, and the less strongly supported clade of Hyacinthoideae Link (56%), a group of species that contains phenolic substances (homoisoflavanoles).

In addition to the classification in four subfamilies, a further species distribution of the subfamilies Hyacinthoideae and Ornithogaloideae into tribes is reflected in the cladogram. Within Hyacinthoideae two clearly separated branches are formed (Figs. 1, 3). The basal branch combines all Indian and African genera south of the Sahara and coincides with the delineation of the tribe Massonieae Baker (67%). The second and more advanced monophyletic clade includes the Mediterranean and Asian genera [= tribe Hyacintheae Dumort.] and is also supported by bootstrap values (78%). Sister to the Hyacinthoideae is the subfamily Ornithogaloideae, which can be further divided into the tribes Dipcadieae Rouy and Ornithogaleae Rouy. However, the relationships are less pronounced in the gene trees (Figs. 1, 4). The Dipcadieae comprise the genera *Pseudogaltonia, Dipcadi, Galtonia, Stellarioides*, and *Albuca* and appear paraphyletic in the analysis, whereas the remaining, mainly Mediterranean genera belong to the tribe Ornithogaleae. If South African *Zahariadia*, which shows affinities to members of *Galtonia*, is excluded from Ornithogaleae, monophyly in this tribe is strongly supported (97%).

The most basal and isolated position in Hyacintheae is occupied by *Barnardia*, a genus with a huge geographical distribution ranging from North

Africa to Japan (Fig. 1). The adjacent clade with *Brimeura, Oncostema, Tractema, Autonoë,* and *Hyacinthoides* is sister to a more advanced clade containing the *Scilla* and *Hyacinthus* alliances. Within *Hyacinthoides,* a division into subgenus *Somera* (Salisb.) Speta consisting of *H. italica* (L.) Rothm., *H. lingulata* (Poir.) Rothm., and *H. aristidis* (Coss.) Rothm. (94%) and subgenus *Hyacinthoides* consisting of *H. nonscripta* (L.) Chouard ex Rothm., *H. hispanica* (Mill.) Rothm., *H. vincentina* (Hoffmanns. & Link) Rothm., and *H. reverchonii* (Degen & Hervier) Speta (85%) is visible (Fig. 3). These two subgenera are characterized by differences in flower morphology, consisting either of a stellate or a campanulate perianth, respectively (Speta, 1987). Furthermore, a well-supported clade with *Hyacinthella, Prospero, Puschkinia, Othocallis, Pfosseria, Hyacinthus, Fessia,* and *Zagrosia* (95%) and a clade with *Chouardia, Nectaroscilla, Schnarfia, Muscari, Bellevalia,* and *Scilla* (84%) are resolved (Fig. 1). There is no bootstrap support for a genus *Chionodoxa.* On the contrary, members of this obsolete genus (*S. nana* (J. A. & J. H. Schultes) Speta, *S. siehei* (Stapf) Speta, *S. albescens* Speta) are intermixed with species of *Scilla* (Fig. 1).

Within Massonieae, a close relationship between *Drimiopsis* and *Ledebouria* is evident (100% bootstrap support), as well as a relationship between *Lachenalia* and *Polyxena* (100%). However, both *Ledebouria* and *Lachenalia* are genera with huge numbers of species, the examination of which still can lead to further differentiation.

Sister to Hyacinthoideae is a clade combining members of the subfamily Ornithogaloideae (Fig. 4). Basal in Ornithogaloideae are members of the tribe Dipcadieae, which accommodates mainly South African genera (*Galtonia, Albuca, Stellarioides, Pseudogaltonia, Dipcadi*). Interestingly, *Galtonia candicans* (Baker) Decne. groups with *Stellarioides* and *Albuca* (86%), whereas *G. viridiflora* Verdoorn and *G. princeps* (Baker) Decne. are combined in one clade with *Zahariadia* (98%) (Fig. 1). Basal in the tribe Ornithogaleae are African species (*Eliokarmos*) followed by the Mediterranean genera *Melomphis* and *Cathissa,* whereas the remaining Mediterranean species are clearly separated (97%) and occur at a more advanced position (e.g., *Loncomelos, Honorius,* and the *Ornithogalum umbellatum* L. and affines).

The next major clade contains species belonging to the subfamily Urgineoideae (Fig. 1). Clearly included in Urgineoideae is the rather peculiar genus *Bowiea* (100%), which lacks leaves as an adult plant except for the bulb scales, and assimilates energy by an intricately branched inflorescence. In this subfamily the basal positions are again occupied by genera from South Africa and Madagascar (*Bowiea, Rhadamanthus, Thuranthos, Ebertia, Urginavia, Karoophila*), and are sister to a clade combining the Mediterranean genera *Urginea* and *Charybdis* (100%).

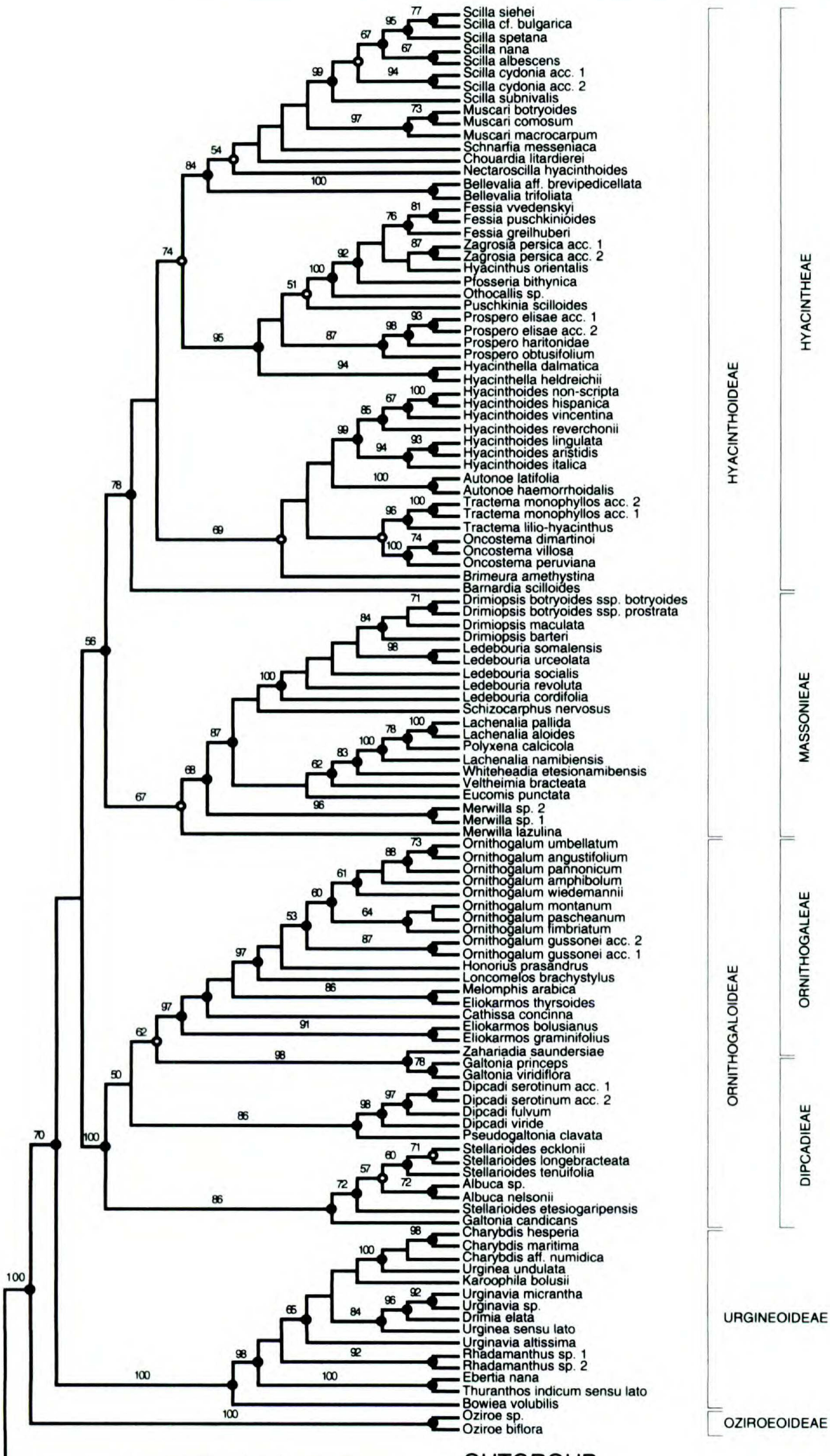
Sister to all Old World Hyacinthaceae is the South American subfamily Oziroëoideae, with the only genus *Oziroë* [= *Fortunatia* Macbr.] (Fig. 1).

Clearly distinct from Hyacinthaceae are the North American bulbous genera *Chlorogalum* and *Camassia,* which form a monophyletic clade (97%) (Figs. 2, 5). Instead, these genera show affinities to the North American family Agavaceae, the East Asian Funkiaceae, and Anthericaceae, which show a worldwide distribution even in the narrow circumscription of Conran (1998a) but with major centers of diversity in Africa, Southeast Asia, and Central and South America. Between this *Chlorogalum–Camassia* clade and Hyacinthaceae, the family Themidaceae, which is endemic to North America, is inserted, although this tree topology is not supported by bootstrap values. More distantly related to Hyacinthaceae are members of the Australian families Lomandraceae, Doryanthaceae, Blandfordiaceae, members of the Asian family Hemerocallidaceae, and of the African, South American, and Laurasian family Amaryllidaceae. Interestingly, *trn*L and *trn*F sequence data place Blandfordiaceae and Doryanthaceae (100%) in close association (Fig. 2). More distantly related are other asparagoid Lilianae like the Laurasian family Convallariaceae, and the North American Nolinaceae. A possible relationship to Alliaceae could not be evaluated since their sequences are too divergent due to extensive deletions and rearrangements in the *trn*L and *trn*F region (data not considered here).

Discussion

Phylogenetic analysis of about 53 genera in Hyacinthaceae and 24 outgroup genera (including *Camassia* and *Chlorogalum*) provides additional data to readdress several critical systematic questions of this controversial plant family.

Scilla clade. Our data strongly support a narrow concept of the genus *Scilla,* as already evident from karyological and morphological data (Speta, 1979, 1986, 1987, 1998a, b). According to our sequence data, the only true *Scilla* species are found in the European and Southwest Asian Mediterranean region, and no direct relatives exist in Africa south of the Sahara (Speta, 1998a). Inclusion of sequence data of the species *S. lazulina* Wild and *S. nervosa* Burch. in a recently published study on


Scilla siehei
Scilla cf. bulgarica
Scilla spetana
Scilla nana
Scilla albescens
Scilla cydonia acc. 1
Scilla cydonia acc. 2
Scilla subnivalis
Muscari botryoides
Muscari comosum
Muscari macrocarpum
Schnarfia messeniaca
Chouardia litardierei
Nectaroscilla hyacinthoides
Bellevalia aff. brevipedicellata
Bellevalia trifoliata
Fessia vvedenskyi
Fessia puschkinioides
Fessia greilhuberi
Zagrosia persica acc. 1
Zagrosia persica acc. 2
Hyacinthus orientalis
Pfosseria bithynica
Othocallis sp.
Puschkinia scilloides
Prospero elisae acc. 1
Prospero elisae acc. 2
Prospero haritonidae
Prospero obtusifolium
Hyacinthella dalmatica
Hyacinthella heldreichii
Hyacinthoides non-scripta
Hyacinthoides hispanica
Hyacinthoides vincentina
Hyacinthoides reverchonii
Hyacinthoides lingulata
Hyacinthoides aristidis
Hyacinthoides italica
Autonoe latifolia
Autonoe haemorrhoidalis
Tractema monophyllos acc. 2
Tractema monophyllos acc. 1
Tractema lilio-hyacinthus
Oncostema dimartinoi
Oncostema villosa
Oncostema peruviana
Brimeura amethystina
Barnardia scilloides
Drimiopsis botryoides ssp. botryoides
Drimiopsis botryoides ssp. prostrata
Drimiopsis maculata
Drimiopsis barteri
Ledebouria somalensis
Ledebouria urceolata
Ledebouria socialis
Ledebouria revoluta
Ledebouria cordifolia
Schizocarphus nervosus
Lachenalia pallida
Lachenalia aloides
Polyxena calcicola
Lachenalia namibiensis
Whiteheadia etesionamibensis
Veltheimia bracteata
Eucomis punctata
Merwilla sp. 2
Merwilla sp. 1
Merwilla lazulina
Ornithogalum umbellatum
Ornithogalum angustifolium
Ornithogalum pannonicum
Ornithogalum amphibolum
Ornithogalum wiedemannii
Ornithogalum montanum
Ornithogalum pascheanum
Ornithogalum fimbriatum
Ornithogalum gussonei acc. 2
Ornithogalum gussonei acc. 1
Honorius prasandrus
Loncomelos brachystylus
Melomphis arabica
Eliokarmos thyrsoides
Cathissa concinna
Eliokarmos bolusianus
Eliokarmos graminifolius
Zahariadia saundersiae
Galtonia princeps
Galtonia viridiflora
Dipcadi serotinum acc. 1
Dipcadi serotinum acc. 2
Dipcadi fulvum
Dipcadi viride
Pseudogaltonia clavata
Stellarioides ecklonii
Stellarioides longebracteata
Stellarioides tenuifolia
Albuca sp.
Albuca nelsonii
Stellarioides etesiogaripensis
Galtonia candicans
Charybdis hesperia
Charybdis maritima
Charybdis aff. numidica
Urginea undulata
Karoophila bolusii
Urginavia micrantha
Urginavia sp.
Drimia elata
Urginea sensu lato
Urginavia altissima
Rhadamanthus sp. 1
Rhadamanthus sp. 2
Ebertia nana
Thuranthos indicum sensu lato
Bowiea volubilis
Oziroe sp.
Oziroe biflora
OUTGROUP
HYACINTHEAE
MASSONIEAE
HYACINTHOIDEAE
ORNITHOGALEAE
DIPCADIEAE
ORNITHOGALOIDEAE
URGINEOIDEAE
OZIROEOIDEAE

sub-Saharan *Scilla* relationships (Stedje, 1998) clearly supports this view. Both species group within the Massonieae clade and show no relationship to *Scilla* sensu stricto and therefore have been named *Merwilla lazulina* (Wild) Speta and *Schizocarphus nervosus* (Burch.) Speta, respectively (Speta, 1998a). Although *Chionodoxa* can be easily discriminated from *Scilla* due to the basal fusion of the tepals and a broadening of the filaments, these morphological characters are overestimated as useful phylogenetic markers. Instead, in phylogenetic reconstructions this character appears to be paraphyletic and seems to have evolved in several lines within the family. Moreover, *Chionodoxa* species can give rise to hybrids when crossed with members of the *Scilla bifolia* L. complex. It has been proposed to include them in *Scilla*, whereas *Puschkinia*, similar to *Chionodoxa* in habit, evidently has other affinities (Speta, 1971, 1976). DNA sequence data place *Puschkinia* in a clade with *Hyacinthella*, *Prospero*, *Othocallis*, *Pfosseria*, *Hyacinthus*, *Zagrosia*, and *Fessia*, clearly different from the *Scilla* relationship (95%) (Fig. 1). However, the phylogenetic signal within the *Scilla* clade is not very strong. Branch lengths differ in only 1 to 4 steps in this clade (Fig. 3). The two accessions of *S. cydonia* Speta even have exactly the same DNA sequences, although they are collected from geographically distant regions (Crete and Karpathos). Re-examination of this relationship with additional molecular data sets could increase the resolution in this clade.

The genus *Muscari* is monophyletic and represents an example of how in Hyacinthaceae even karyotype differences can be overestimated (Garbari & Greuter, 1970). Monophyly in this clade could support the idea of a broader genus concept in *Muscari* (Speta, 1982, 1989) instead of dividing this genus into the genera *Muscari*, *Leopoldia*, and *Muscarimia*, represented in our analysis by *Muscari botryoides* (L.) Mill., *M. comosum* (L.) Mill., and *M. macrocarpum* Sweet, respectively (Fig. 1). Whereas Garbari recognized these genera as distinct from each other due to karyotypic differences, Speta (1982, 1989), Bentzer (1973), and Davis and Stuart (1984) found transitions in character expression between different species and therefore suggested they be treated only as subgenera.

Fessia clade. Similarities in the karyotypes of *Chouardia* Speta [= *Scilla litardierei* Breistr.] and *Hyacinthella* as well as morphologies (locules with two ovules side by side, terete scape, dense raceme) have led to the speculation of a closer relationship (Speta, 1981). However, Persson and Wendelbo (1982) supported the view that the similarity in the karyotypes is probably only secondary, constituting a case of convergent evolution within the *Scilla* sensu lato lineage as well as the lineage to present *Hyacinthella* species. In our analysis, *Hyacinthella* clearly belongs to the clade *Fessia–Zagrosia–Hyacinthus–Pfosseria–Othocallis–Puschkinia–Prospero* (95%), and not to the *Scilla* relationship (Fig. 1). However, the relative position of *Hyacinthella* within this clade is not stable due to a basal polytomy in this group (Fig. 3). Unfortunately, *Alrawia*, a genus that could show affinities to *Hyacinthella* and therefore could shed light on this relationship, was not available for this study. The IGS sequences of the two *Hyacinthella* species contain a characteristic 5 bp deletion, which they share with Oziroëoideae, Ornithogaloideae, the African genera *Eucomis*, *Veltheimia*, and the *Lachenalia/Polyxena* clade: this may suggest the motif represents an ancient element. The existence of ancient elements in DNA sequences of species belonging to different groups sometimes results in weakly supported tree topologies. This is also the case with the position of *Hyacinthella*, which in some trees showed a different position within this clade (data not shown). Although the habit of problematic *Puschkinia* closely resembles that of *Scilla bifolia*, phylogenetic reconstructions position it clearly distinct from *Scilla* sensu stricto. Additionally, a suite of morphological characters also clearly separates *Puschkinia* from *Scilla*. The corolla and corona, the *Fessia*-like ovary, seeds with a sarcotesta, bulbs with vaginate scales, and chromosome number ($2n = 10$) are all characters discriminating *Puschkinia* from *Scilla*. A similar chromosome number in *Fessia* ($2n = 10$) suggests a possible relationship, but Greilhuber and Speta (1976) demonstrated karyological differences. A closer relationship with *Muscari* (Wunderlich, 1937) is refuted by our DNA sequence data. In *Fessia*, two species complexes have been proposed: (1) the *S. hohenackeri* group and (2) the *S. bisotunensis* relationship (Speta, 1981). Sev-

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Figure 1. One of the most parsimonious successively weighted trees showing the relationships within Hyacinthaceae. Bootstrap percentages are indicated above the branches. Subfamilial and tribal limits sensu Speta (1998a, b) are indicated on the right border. Nodes strongly supported by the jackknife algorithm (node score > 0.63) are indicated by solid circles. Open circles indicate nodes that are weakly supported (node score > 0.5 but < 0.63).

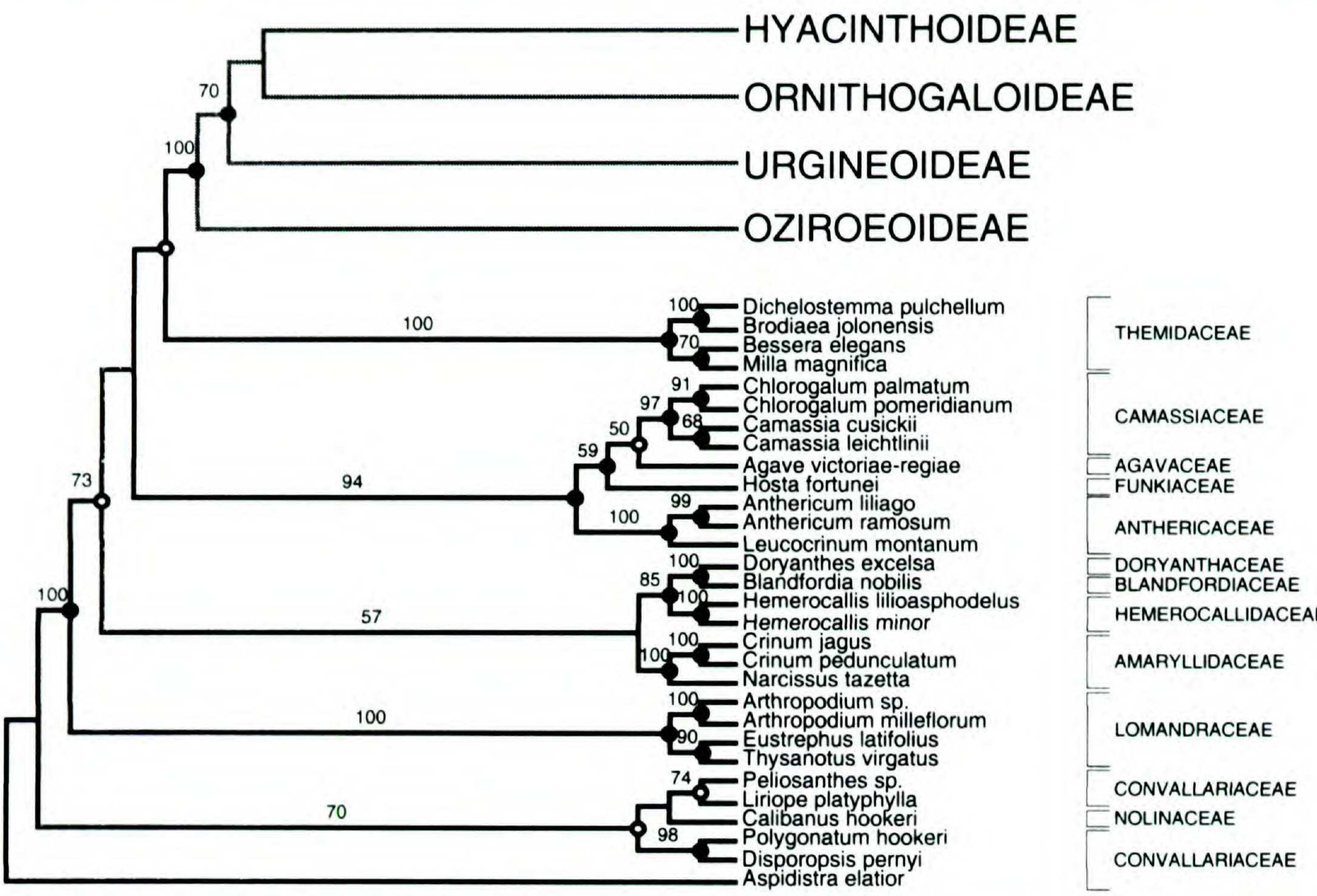


Figure 2. Second part of one of the most parsimonious successively weighted trees showing the outgroup relationships. Bootstrap percentages are indicated above the branches. Nodes strongly supported by the jackknife algorithm (node score > 0.63) are indicated by solid circles. Open circles indicate nodes that are weakly supported (node score > 0.5 but < 0.63).

eral character anomalies exist such as hypogeous cotyledons in *F. greilhuberi* (Speta) Speta but epigeous ones in all other species examined, or a karyotype in *F. gorganica* (Speta) Speta of $2n = 18$ rather than the typical $2n = 10$: these warrant further research in this clade. The close nesting of *Zagrosia* and *Hyacinthus* is particularly unexpected. DNA sequence data of two other *Hyacinthus* species, *H. litwinowii* E. Czern. and *H. transcaspicus* Litw. from Turkmenistan and northeast Iran, respectively, could address the position of *Hyacinthus* in this clade. Similarities in the karyotypes of *Zagrosia* and *Bellevalia* (large chromosomes; $x = 4$; Speta, 1974, 1981: 169) suggest a possible relationship, but no indication of such emerges from the molecular data. The strong separation of *Othocallis* and *Scilla* sensu stricto is somewhat surprising, indicating that morphological and karyological differences may have been underestimated so far.

Hyacinthoides clade. Morphological and karyological evidence do not support a relationship between *Autonoë* and *Hyacinthoides* as suggested by their close association in the cladogram (Figs. 1, 3). Although combined in a separate branch in the molecular analysis, there is no bootstrap support for a possible direct relationship (Fig. 1). Basal to this clade is a cluster with *Brimeura, Oncostema,* and *Tractema,* a relationship already suggested on morphological and karyological grounds (Speta, 1987). Although bootstrap support for this relationship is not very high, closer affinity is likely, since trees constructed from *mat*K sequences directly connect *Brimeura* with *Tractema* (Pfosser, unpublished data). Tree topology clearly corroborates the division of *Hyacinthoides* into subgenera *Somera* and *Hyacinthoides,* a position not shared by Ortiz and Rodríguez-Oubiña (1996).

As in *Hyacinthella, Barnardia,* the most basal member of Hyacinthoideae, also contains sequence elements shared by different groups like *Lachenalia, Polyxena,* and *Dipcadi,* again indicating an ancient origin for *Barnardia.* Evidence relating *Barnardia* to *Prospero* Speta (1986, 1993) is based on the dysploid series of chromosome numbers (*Prospero,* $x = 4, 5, 6, 7$, and *Barnardia,* $x = 8, 9$), and that both are autumn-flowering genera with similar habit. This relationship is not supported by the molecular data.

Massonieae clade. All South African genera cluster together in tribe Massonieae. This is surprising since they appear morphologically hetero-

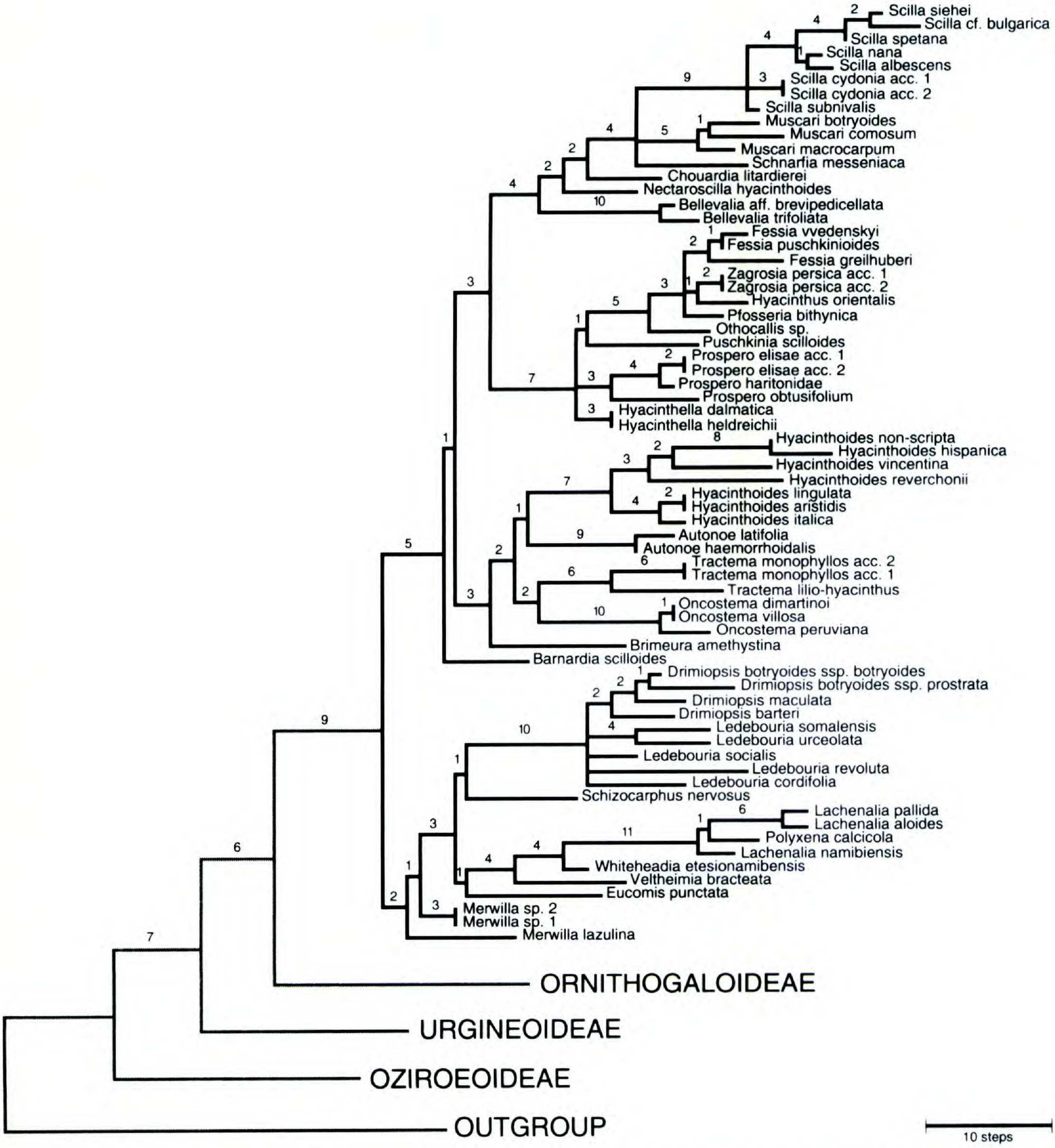


Figure 3. Phylogram showing the relationships within subfamily Hyacinthoideae. Fitch branch lengths are indicated above the branches. For bootstrap percentages compare with Figure 1.

geneous when considering *Eucomis*, *Veltheimia*, *Lachenalia*, *Ledebouria*, and *Merwilla*. It would be interesting to more thoroughly investigate the genera *Lachenalia* and *Polyxena*. *Lachenalia* with almost 100 species was split into several genera by Salisbury (1866). Although only three species were analyzed by us, our data at least suggest that there might be enough variability to warrant further splitting of this genus. If the generic status of *Polyxena* is maintained, then at least two relationships are indicated: the *Lachenalia namibiensis* W. F. Barker and the *L. aloides* (L. f.) Engl./*L. pallida* Aiton relationships. In contrast to our evidence for close relationship between *Lachenalia* and *Polyxena*, Müller-Doblies and Müller-Doblies (1997) placed these two genera in the different subtribes Lachenaliinae and Massoniinae, respectively. In their classification, *Lachenalia* and *Veltheimia* belong to the subtribe Lachenaliinae, whereas *Whiteheadia*, which in our analysis inserts between these two genera, belongs to subtribe Massoniinae. A close relationship between *Ledebouria* and *Drimiopsis* [= subtribe Ledebouriinae sensu U. & D. Müller-Doblies] is supported by our molecular data (100%

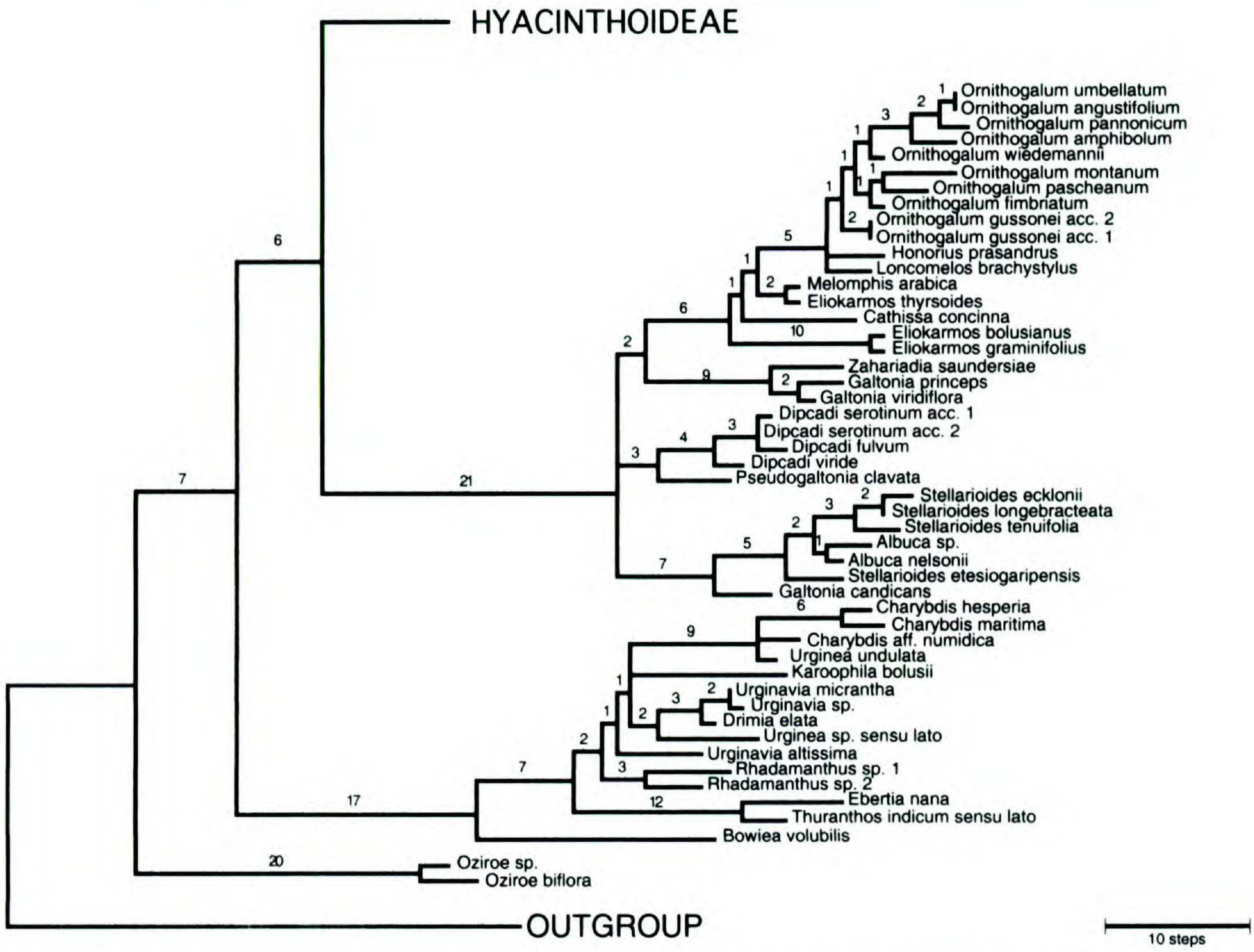


Figure 4. Phylogram showing the relationships within the subfamilies Ornithogaloideae, Urgineoideae, and Oziroëoideae. Fitch branch lengths are indicated above the branches. For bootstrap percentages compare with Figure 1.

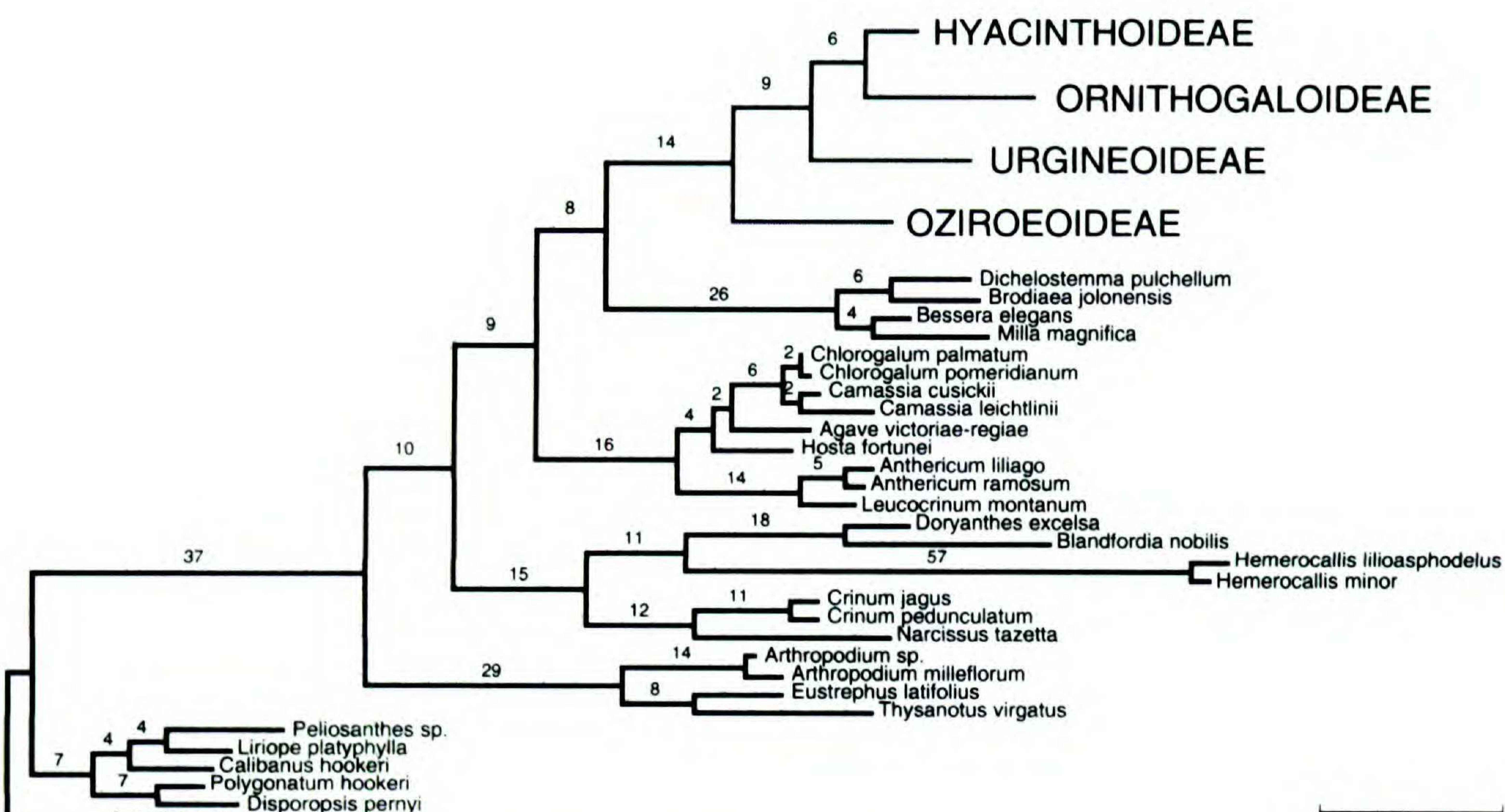


Figure 5. Phylogram showing the outgroup relationships. Fitch branch lengths are indicated above the branches. For bootstrap percentages compare with Figure 2.

bootstrap support in Fig. 1). However, *Resnova*, a third genus included in Ledebouriinae, was not available for sequencing. How far the generic combinations of Jessop (1975) within Massonieae are paralleled by molecular data remains to be seen, especially since *Polyxena* and *Lachenalia* are in close neighborhood in our analysis. *Daubenya*, *Androsiphon*, and *Amphisiphon* share many characters with *Whiteheadia* and therefore would no doubt insert in the same branch. However, their individual positions as distinct genera should probably be reevaluated since various prominent features of corolla and filaments might have been overestimated so far. Stedje (1998) published a study examining the relationships of sub-Saharan *Scilla* sensu lato species, which revealed a close relationship between *Ledebouria* and *Drimiopsis*, whereas the generic status of other members of *Scilla* sensu lato was not resolved. Inclusion of her DNA sequence data in our analysis clearly demonstrated a close relationship of the South African species to *Ledebouria* and *Drimiopsis*, but no affinity to the Mediterranean species.

Less clear are the generic relationships within the two major branches consisting of the subfamilies Ornithogaloideae and Urgineoideae.

Ornithogaloideae clade. The old Linnaean genus *Ornithogalum*, a heterogeneous assemblage of numerous species, represents a big taxonomic problem and still awaits its distribution into more natural genera. This unsatisfactory situation may partly inflate the analysis, since taxon sampling in Ornithogaloideae is less complete than in Hyacinthoideae. Nevertheless, the 30 species investigated in this study form a monophyletic group (100% bootstrap support in Fig. 1). Taxon sampling should be further increased as soon as a clearer view of this large genus is available. One distinct branch, combining *Stellarioides* and *Albuca*, one with *Dipcadi* and *Pseudogaltonia*, and one with the rest of the subfamily are visible (Fig. 1). In the phylogram, a polytomy is indicated with these three clades arising from a single node (Fig. 4). Within Ornithogaleae, *Cathissa* from the southwest Mediterranean and *Eliokarmos* from South Africa appear as basal genera, while the generitype *O. umbellatum* L. occupies a rather derived position (Fig. 4). Based on DNA sequence data, a proposed relationship between the *Ornithogalum longebracteatum* Jacq. group [= *Stellarioides*] and *Ornithogalum* subg. *Beryllis* [= *Loncomelos*] (Baker, 1873) can be clearly refuted. The first group of species is in close vicinity to *Albuca* and belongs to *Stellarioides*. Originally included under *Ornithogalum*, a closer relationship of this genus with *Albuca* was noticed by Obermeyer (1978). Once again, only a few indications of a direct relationship between Mediterranean and South African genera are recognizable. Similar to the *Scilla* clade, only short branches separate *Ornithogalum* species from each other. Again, molecular data sets with higher variability could probably increase the resolution within this *Ornithogalum* clade. Preliminary *trn*L and *trn*F sequence data of the *Ornithogalum montanum* Ten. sensu lato relationship does suggest that sequence variation in this region may suffice to describe different geographical races as they occur in the Aegean region (Pfosser, unpublished data).

Urgineoideae clade. Obscure relationships similar to those in Ornithogaloideae also exist in the large subfamily Urgineoideae, with *Bowiea* providing a historic example. Takhtajan (1997) questioned its position in Hyacinthaceae, concluding that this genus probably should be included somewhere else. However, it appears clear now from Figure 1 that *Bowiea* has to be included in subfamily Urgineoideae after misplacement in Asphodeloideae–Eriosperminae (Engler, 1887) and in Liliaceae–Bowieae (Hutchinson, 1934). Even more problematic is the taxonomic history of Mediterranean squill. This old medicinal plant was first treated as *Scilla maritima* (Linnaeus, 1753), then as *Urginea maritima* aggregate (Steinheil, 1834), later separated from *Urginea* as *Squilla* (Steinheil, 1836). It has even been included in *Drimia* (Jessop, 1977; Stearn, 1978; Stedje, 1987). Both Speta (1998a) and Steinheil (1836) were convinced of an independent status of the *Urginea maritima* relationship, which consequently led to the introduction of the genus *Charybdis* (Speta, 1998a). In Figure 1, the Mediterranean squills are at least in the vicinity of a true member of *Urginea* (*U. undulata* (Desf.) Steinh.). The number of species investigated in this clade is not sufficient to lead to an adequate description of this subfamily; however, the results thus obtained do provide valuable anchor points for additional sampling.

Oziroeoideae clade. The last subfamily in Hyacinthoideae contains the South American genus *Oziroë* [= *Fortunatia*] and occupies an isolated basal position in Hyacinthaceae. This indicates an ancient split between Old World and New World taxa, resulting in two independent evolutionary lineages. If there are direct relatives to *Oziroë* existing somewhere in Africa, they remain to be found.

Outgroup. Enough evidence has accumulated to demonstrate that the North American genera *Camassia* and *Chlorogalum* can no longer be included in Hyacinthaceae (Figs. 2, 5). Our *trn*L and *trn*F sequence data, *rbc*L sequence data (Fay & Chase,

1996; Chase et al., 1995), as well as serological data (Cupov & Kutjavina, 1981), all point to a relationship of these genera to Agavaceae, Funkiaceae, and Anthericaceae and not to Hyacinthaceae. Recognizing these genera as a separate family Camassiaceae as proposed by Cupov (1994) should await evaluation of all available data. For now we concur, since this proposal offers the most appropriate perspective on these genera. At least *Hosta*, *Camassia*, and *Chlorogalum* share a bimodal karyotype typical for Agavaceae. However, Agavaceae represent a paraphyletic assemblage in their present circumscription as suggested by nuclear ITS sequence data (Bogler & Simpson, 1996). Recently, now that increasing *rbc*L data has accumulated and been phylogenetically evaluated, major rearrangements among the Liliaceae sensu lato, Anthericaceae, and other monocot families have been attempted. Kubitzki (1998) tried to incorporate molecular information in his treatment of monocots in volume III of his *The Families and Genera of Vascular Plants*. Chase et al. (1996) previously noted that Amaryllidaceae, Alliaceae, and Lomandraceae are probably sister families to Hyacinthaceae. Moreover, Themidaceae have been shown to be in close vicinity to the Hyacinthaceae (Fay & Chase, 1996), a view supported also from *trn*L and *trn*F data (Fig. 2). Since the position of *Chlorogalum* and *Camassia* also had to be investigated, we included a few less well-known potential relatives in our analysis. Somewhat surprising in our analysis is the position of the family Amaryllidaceae, which is sister to a clade combining *Hemerocallis* and *Doryanthes* and *Blandfordia*. Since an affinity of Doryanthaceae to Phormiaceae was suggested (Dahlgren et al., 1985), and the Hemerocallidaceae were recently coalesced with Phormiaceae and are no longer monogeneric (Clifford et al., 1998), this clade becomes more understandable. Although bound by relatively long branches (Fig. 5), *Doryanthes* and *Blandfordia*, both monotypic for their respective families (Dahlgren et al., 1985), combine in one clade (100% bootstrap support in Fig. 2). Adjacent to this clade, *Arthropodium*, *Thysanotus*, and *Eustrephus* also combine (100% bootstrap support in Fig. 2). Support for this comes from *rbc*L sequence data (Chase et al., 1996; Conran, 1998b), with these species combined under Lomandraceae.

Deep branching points of all subfamilies except Hyacinthoideae are characterized by long branches of 21 steps (Ornithogaloideae), 17 steps (Urgineoideae), and 20 steps (Oziroeoideae), indicating a high number of nucleotide changes before speciation within the subfamilies occurred (Fig. 4). This could either suggest that the nucleotide substitution rate in this family was higher during a primary radiation than subsequent ones, or that sampling bias occurred because of extinction events, or that primary radiation occurred slowly. The presence of a few isolated genera like *Brimeura*, *Barnardia*, *Merwilla*, and *Bowiea* at basal positions in some subfamilies, however, could suggest extinction events. If other basal genera still exist, then they probably will be found among the Urgineoideae or Ornithogaloideae in South Africa or Madagascar.

Most genera in this analysis grouping together also show a sympatric occurrence. While true for Hyacintheae or Massonieae in general, *Galtonia* and *Zahariadia* from eastern South Africa are also sympatric (Obermeyer, 1978; Hilliard & Burtt, 1988), as are *Tractema*, *Oncostema*, *Brimeura*, and *Hyacinthoides* from the western Mediterranean, as well as outgroup genera like *Blandfordia* and *Doryanthes*. Similarly, that molecular data reflect sympatric relationships was already noted for *Crinum* species (Fangan & Nordal, 1993). This in turn suggests that environmental influences leading to convergent evolution or reversals of morphological characters after species migration should be cautiously re-examined with respect to systematic research. To address this, it would be necessary to evaluate morphological characters that are not or only to a small extent influenced by environmental constraints. It is still not clear if molecular data, especially noncoding DNA regions, are absolutely free of biases resulting from convergent evolution, which could inflate the significance of the analysis. Recently, it has been shown that even noncoding DNA, free of functional constraints, still can show convergent evolution due to specific sequence motifs, which can lead to preferred sites of mutations (Morton, 1995). It should also be noted that the most advanced positions in each clade are generally occupied by genera containing numerous species (*Fessia*, *Scilla*, *Hyacinthoides*, *Drimiopsis* and *Ledebouria*, *Ornithogalum*, *Dipcadi*, *Charybdis*), indicating a process of active evolution. Conversely, basal positions of clades usually contain genera with only a few species in relic areas like *Bowiea* in southern and eastern Africa, *Galtonia* and *Zahariadia* in eastern South Africa, *Pseudogaltonia* in western South Africa, *Merwilla* in South Africa, *Chouardia* in the Dalmatian region, *Nectaroscilla* in the eastern Mediterranean, and *Brimeura* in the western Mediterranean. Exceptions to this trend are: (1) the large genus *Lachenalia*, with a rather confined radiation south of the Sahara; and (2) *Barnardia*, a basal genus with a huge disjunct distribution extending from northwest Africa to southeast Asia. Alternately, *Barnardia* could be viewed as the

only extant genus of a largely extinct clade, as indicated by its isolated basal position in subfamily Hyacinthoideae.

Involvement of species from South Africa, South America, and Madagascar in the basal branchings of subfamilies and tribes is indicative of southern, Gondwanic, origin of the Hyacinthaceae. Direct migration between South America, Africa, Madagascar, and India was possible until the mid Cretaceous (ca. 100 m.y. BP). Similar radiation patterns are known elsewhere, such as fossil evidence for sauropods in the Upper Cretaceous of Madagascar, with the genus *Laplatasaurus* reported in India and Madagascar, as well as South America (Charig, 1973). Since there is no evident relationship of North American *Chlorogalum* and *Camassia* species to the Hyacinthaceae, species diversification or migration to different continents perhaps did not start before Laurasia and Gondwanaland were separated in early Cretaceous. At this time, South America was still connected to Africa, but no land bridge existed between South and North America. Species distribution within Massonieae in Africa only south of the Sahara, and in the Indian subcontinent (*Ledebouria*, *Drimiopsis*), further suggests that diversification occurred when India was still connected to southern Africa, not only via species migration to northern Africa and Eurasia. The distribution pattern of members of the tribe Hyacintheae in the Mediterranean region and in Eurasia, but not in North America, suggests that diversification in this tribe began later, when North America was already clearly separated from Eurasia. The appearance of *Barnardia* at the most basal position of Hyacintheae indicates an early origin of evolution for this genus, making it a candidate for first colonization of the Mediterranean region. Its continental distribution from north Africa to east Asia, even to Japan, is a typical radiation pattern for an old taxon. This would explain why no members of the Hyacinthaceae are found in North America, although climatic conditions are suitable. An unrelated origin of the Camassiaceae apart from the Hyacinthaceae moreover avoids such an unusual disjunction between the Camassiaceae, endemic to North America, and monotypic *Oziroë* in Chile. Similarily, the paradox of range disjunction between Alliaceae and Brodiaeae in North America, yet the rest of the Alliaceae being mainly South American, was recently elucidated by the phylogenetic positioning of Brodiaeae apart from Alliaceae. To retain a monophyletic Alliaceae, the family Themidaceae was resurrected for this tribe (Fay & Chase, 1996). For the entire order Asparagales, West Gondwanaland most likely was the primary site of evolution. Family members close to Asphodelaceae and Phormiaceae seem to have reached Australasia early, whereas elements related to Asparagaceae, Asphodelaceae, and Amaryllidaceae–Hemerocallidaceae suggest old connections between Africa and South America. The Agavaceae alliance of subhumid and arid regions of North America presumably represent an ancient Laurasian group (Raven & Axelrod, 1974). Similar interfamilial relationships have already been proposed by Fay and Chase (1996). The Alliaceae alliance, which in their study shows affinities to Amaryllidaceae, has been excluded in our analysis since their sequences are too divergent due to massive deletions and rearrangements in the *trn*L and *trn*F regions: their inclusion would have inflated the phylogenetic signal in the calculations (data not shown).

For no plant family is it more true than for Hyacinthaceae that the interpretation of single morphological characters resulted in highly erratic classifications when delineating tribal and subfamilial relationships. No character, from bulb morphology to pistils or seeds, or even karyological data, has proved to be reliable. Therefore, it was necessary to assemble all available information for proper classification. Four subfamilies were best distinguished by their secondary metabolites and by the presence or absence of nuclear protein crystals. Assessment of relationships based on molecular data offers the considerable advantage that a priori biases of relative character value are largely avoided. Comparing groups at generic and higher hierarchical levels as classified by Speta (1998a, b) with cladistic analysis of molecular data was clearly warranted. It was more than a surprise when sequence data confirmed not only the higher-level classification but also most generic groupings. Outside of Scilleae, only *Bowiea* and *Schizobasis* had to be included in Hyacinthaceae, and exclusion of Camassiaceae finally resulted in a monophyletic family Hyacinthaceae. The Hyacinthaceae obviously represent the final products of a lineage showing affinities to Themidaceae, Anthericaceae, and Amaryllidaceae. This cladistic analysis results in significant progress in the splitting of the Linnaean genera *Scilla* and *Hyacinthus* into more homogeneous genera. *Ornithogalum* and some complexes within Urgineoideae still await further taxonomic resolution. However, the molecular data presented here serve as valuable starting points toward identifying critical taxa or for the evaluation of additional nonmolecular characters.

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Appendix 1. Species analyzed, arranged alphabetically by families, including collector's data and EMBL data library accession numbers. ° Vouchers for all species sequenced in this study are deposited in LI unless other herbarium designations are given. * Species, for which DNA sequences have been produced directly from herbarium specimens. § Publication, in which trnL/trnF sequences for this taxon were first cited.

Species	Family	Locality	Voucher°	EMBL acc. no. Intron	EMBL acc. no. IGS	Literature citation§
Agave victoriae-regiae T. Moore	Agavaceae	Mexico: Nueva Leon, Huasteca Canyon	Schatzl AG02	AJ232447	AJ232570	this paper
Crinum jagus (Thomson) Dandy	Amaryllidaceae	Cameroon: 3 km E of Ngaoundere	I. Nordal 952 (O)	X74729	X74580	Fangan et al., 1994
Crinum pedunculatum R. Br.	Amaryllidaceae	Australia: N.S.W., Highway btw. Coopernook and Taree, 20 m	Speta H422	AJ232438	AJ232561	this paper
Narcissus tazetta L.	Amaryllidaceae	Italy: Capri, below Villa Javi	Kiehn AM01	AJ232439	AJ232562	this paper
Anthericum liliago L.	Anthericaceae	Austria: Niederösterreich	Pfosser LI04	AJ232444	AJ232567	this paper
Anthericum ramosum L.	Anthericaceae	Austria: Burgenland	Pfosser LI05	AJ232445	AJ232568	this paper
Leucocrinum montanum Nutt. ex A. Gray	Anthericaceae	USA: Colorado	Chase 795 (K)	AF117003	AF117031	Meerow et al., 1999
Blandfordia nobilis Sm.	Blandfordiaceae	Australia: N.S.W., Royal N.P. near Heathcote, 180 m	Speta H416	AJ232441	AJ232564	this paper
Camassia cusickii Lindl.	Camassiaceae	cult. B. G. Linz	Speta H223	AJ232450	AJ232573	this paper
Camassia leichtlinii S. Wats.	Camassiaceae	cult. ex B. G. Marburg (69/84)	Pfosser H231	AJ232451	AJ232574	this paper
Chlorogalum palmatum (Lindl.) Kunth	Camassiaceae	USA: California, Highway 128, 300 m	Quibell H260	AJ232448	AJ232571	this paper
Chlorogalum pomeridianum (Lindl.) Kunth	Camassiaceae	USA: California, Rohnert Park	Quibell H012	AJ232449	AJ232572	this paper
Aspidistra elatior Blume	Convallariaceae	cult., origin unknown	Chase 833 (K)	AF117016	AF117044	Meerow et al., 1999
Disporopsis pernyi (Hua) Diels	Convallariaceae	China: Jiangsu Province	Chase 493 (K)	—	—	Chase, unpublished
Liriope platyphylla F.T. Wang & T. Tang	Convallariaceae	cult., origin unknown	Chase 131 (NCU)	AF117009	AF117038	Meerow et al., 1999
Peliosanthes sp.	Convallariaceae	Indonesia: Borneo	Chase 847 (K)	AF117006	AF117034	Meerow et al., 1999
Polygonatum hookeri Baker	Convallariaceae	Nepal	Chase 492 (K)	AF117010	AF117036	Meerow et al., 1999
Doryanthes excelsa Correa	Doryanthaceae	Australia: N.S.W., Brisbane Water N. P., near Mt. White, 150 m	Speta H415	AJ232440	AJ232563	this paper
Hosta fortunei L.H. Bailey	Funkiaceae	cult. ex B. G. Nantes (1168/97)	Pfosser H433	AJ232446	AJ232569	this paper
Hemerocallis lilioasphodelus L.	Hemerocallidaceae	cult. ex B. G. Bonn (410/97)	Pfosser H435	AJ232442	AJ232565	this paper
Hemerocallis minor Mill.	Hemerocallidaceae	cult. ex B. G. Bonn (8411/97)	Pfosser H434	AJ232443	AJ232566	this paper
Albuca nelsonii N.E. Br.	Hyacinthaceae	cult. B. G. Vienna	Pfosser H062	AJ232468	AJ232591	this paper

Appendix 1. Continued.

Species	Family	Locality	Voucher°	EMBL acc. no. Intron	EMBL acc. no. IGS	Literature citation§
Albuca sp.	Hyacinthaceae	Guinea: Moyenne Guinée, NW of Timbo	Ebert & Bangoura H204	AJ232467	AJ232590	this paper
Autonoe haemorrhoidalis (WEBB & BERTH.) SPETA	Hyacinthaceae	Spain: Teneriffa	Klenner H160	AJ232518	AJ232641	this paper
Autonoe latifolia (WILLD.) SPETA	Hyacinthaceae	Morocco: Agadir	Ehrendorfer H015	AJ232517	AJ232640	this paper
Barnardia scilloides LINDL.	Hyacinthaceae	Japan, ex B. G. Kanagawa (214/94)	Pfosser H025	AJ232509	AJ232632	this paper
Bellevalia aff. brevipedicellata TURRILL	Hyacinthaceae	Greece: Crete, Ep. Selinou, Gavdos	Jahn H214	AJ232547	AJ232670	this paper
Bellevalia trifoliata KUNTH	Hyacinthaceae	Greece: Rhodos, Faliraki	Speta H052	AJ232548	AJ232671	this paper
Bowiea volubilis HARVEY EX J.D. HOOKER	Hyacinthaceae	S Africa, cult. B. G. Vienna	Pfosser H222	AJ232454	AJ232577	this paper
Brimeura amethystina (L.) CHOUARD	Hyacinthaceae	cult. ex B. G. Tallinn (237/80)	Pfosser H225	AJ232510	AJ232633	this paper
Cathissa concinna (SALISB.) SPETA	Hyacinathaceae	Spain: Càceres, Las Hurdes	Raus H047	AJ232481	AJ232604	this paper
Charybdis aff. numidica (JORD.) SPETA	Hyacinthaceae	Greece: Crete, Moni Gouvernetou	Weigl H003	AJ232464	AJ232587	this paper
Charybdis hesperia (WEBB & BERTH.) SPETA	Hyacinthaceae	Spain: Fuerteventura, Betancuria, 500 m	Skala H069	AJ232465	AJ232588	this paper
Charybdis maritima (L.) SPETA sensu stricto	Hyacinthaceae	Spain: central Spain	Speta H319	AJ232466	AJ232589	this paper
Chouardia litardierei (BREISTR.) SPETA	Hyacinthaceae	Croatia: Dalmatia, cult. B. G. Vienna	Pfosser H230	AJ232541	AJ232664	this paper
Dipcadi fulvum (CAV.) WEBB. & BERTH.	Hyacinthaceae	Spain: Canar. Island, Lanzarote, NW of Orzola, Playa de la Canteria	Tod H207	AJ232479	AJ232602	this paper
Dipcadi serotinum (L.) MEDICUS acc. 1	Hyacinthaceae	Spain: Mini Hollywood, N of Almeria	Stech H061	AJ232477	AJ232600	this paper
Dipcadi serotinum (L.) MEDICUS acc. 2	Hyacinthaceae	Portugal: Nazaré	Scheiblreiter H336	AJ232478	AJ232601	this paper
Dipcadi viride (L.) MOENCH sensu lato	Hyacinthaceae	Senegal: Région de Fatick, Mbam, 4 km E of Foundiougne	Ebert & Bangoura H365	AJ232476	AJ232599	this paper
Drimia elata JACQ.	Hyacinthaceae	Zimbabwe: 19 km E of Chevy	Nordal 2501	Z99135	Z99136	Stedje, 1998
Drimiopsis barteri BAKER	Hyacinthaceae	Tanzania: Iringa District, Ruaha Natl. Park, 3.5 km N of Msembe	A. Bjornstad 1158	Z99137	Z99138	Stedje, 1998

Appendix 1. Continued.

Species	Family	Locality	Voucher°	EMBL acc. no. Intron	EMBL acc. no. IGS	Literature citation§
Drimiopsis botryoides BAKER subsp. *botryoides*	Hyacinthaceae	Tanzania: Uzaramo District, Mbudya Island	Nordal 1600	Z99139	Z99140	Stedje, 1998
Drimiopsis botryoides BAKER subsp. *prostrata* B. STEDJE	Hyacinthaceae	Tanzania: Iringa District, at the foot of Image Mt.	I. Bjornstad 780	Z99141	Z99142	Stedje, 1998
Drimiopsis maculata LINDLEY	Hyacinthaceae	cult. LI	Speta H002	AJ232502	AJ232625	this paper
Ebertia nana (OYEWOLE) SPETA	Hyacinthaceae	Guinea: Moyenne Guinée, SW of Labé	Ebert & Bangoura H406	AJ232456	AJ232579	this paper
Eliokarmos bolusianus (BAKER) SPETA	Hyacinthaceae	R. South Africa: Graaff-Reinet, 3–4 km from Murragsburg road, 950 m	Müller-Doblies H183	AJ232484	AJ232607	this paper
Eliokarmos graminifolius (THUNB.) SPETA sensu lato	Hyacinthaceae	R. South Africa	Müller-Doblies H141	AJ232485	AJ232608	this paper
Eliokarmos thyrsoides (JACQ.) RAF.	Hyacinthaceae	R. South Africa, cult. B. G. Vienna (ex B. G. Kirstenbosch)	Pfosser H404	AJ232483	AJ232606	this paper
Eucomis punctata (THUNB.) L'HERIT.	Hyacinthaceae	cult. LI	Speta H221	AJ232500	AJ232623	this paper
Fessia greilhuberi (SPETA) SPETA	Hyacinthaceae	cult. B. G. Vienna	Pfosser H013	JA232534	AJ232657	this paper
Fessia puschkinioides (E. REGEL) SPETA	Hyacinthaceae	Tadschikistan: Hissar Mts., 60 km E of Duschanbe	Fritsch H211	AJ232536	AJ232659	this paper
Fessia vvedenskyi (PAZIJ) SPETA	Hyacinthaceae	Uzbekistan: 20 km NE of Schargun	Speta H234	AJ232535	AJ232658	this paper
Galtonia candicans (BAKER) DECNE.	Hyacinthaceae	cult. LI	Speta H233	AJ232472	AJ232595	this paper
Galtonia princeps (BAKER) DECNE.	Hyacinthaceae	South Africa, ex B. G. Harrogate (499/97)	Pfosser H379	AJ232474	AF232597	this paper
Galtonia viridiflora VERDOORN	Hyacinthaceae	South Africa, ex B. G. Harrogate (500/97)	Pfosser H378	AJ232473	AJ232596	this paper
Honorius prasandrus (GRISEB.) HOLUB	Hyacinthaceae	Turkey: Uludag	Mertens H048	AJ232486	AJ232609	this paper
Hyacinthella dalmatica (BAKER) CHOUARD	Hyacinthaceae	Croatia: Dalmatia	Gutermann H297	AJ232526	AJ232649	this paper
Hyacinthella heldreichii (BOISS.) CHOUARD	Hyacinthaceae	Turkey: Sertavul Pass	Markus H210	AJ232527	AJ232650	this paper

Appendix 1. Continued.

Species	Family	Locality	Voucher°	EMBL acc. no.		Literature citation§
				Intron	IGS	
Hyacinthoides aristidis (Coss.) Rothm.	Hyacinthaceae	Algeria: near Yakouren, Djurdjura	Schneider H065	AJ232521	AJ232644	this paper
Hyacinthoides hispanica (Mill.) Rothm.	Hyacinthaceae	Spain: Provincia de Cádiz, 6 km S of Ubrique	Voglmayr H338	AJ232525	AJ232648	this paper
Hyacinthoides italica (L.) Rothm.	Hyacinthaceae	France: Alpes Maritimes (ex B. G. Neuchatel 71/84)	Pfosser H300	AJ232519	AJ232642	this paper
Hyacinthoides lingulata (Poir.) Rothm.	Hyacinthaceae	Tunisia: btw. Nefza and Ain Sebaa	Koenen H066	AJ232520	AJ232643	this paper
Hyacinthoides non-scripta (L.) Chouard ex Rothm.	Hyacinthaceae	France: Maquere sur la route N-823 a 12 km du Man (ex B. G. Neuchatel 145/83)	Pfosser H235	AJ232524	AJ232647	this paper
Hyacinthoides reverchonii (Degen & Hervier) Speta	Hyacinthaceae	Spain: Provincia de Jaén, Parque Nacional Sierra de Cazorla	Voglmayr H307	AJ232523	AJ232646	this paper
Hyacinthoides vincentina (Hoffmanns. & Link) Rothm.	Hyacinthaceae	Portugal: Cabo de Sao Vincente (Vila do Bispo)	Scheiblreiter H305	AJ232522	AJ232645	this paper
Hyacinthus orientalis L.	Hyacinthaceae	Romania: Greci cult.	Speta H067	AJ232539	AF232662	this paper
Karoophila bolusii Müller-Doblies ined.	Hyacinthaceae	—	Müller-Doblies H182	AJ232462	AJ232585	this paper
Lachenalia aloides (L. f.) Engl.	Hyacinthaceae	cult. B. G. Vienna	Pfosser H159	AJ23508	AJ232631	this paper
Lachenalia namibiensis W.F. Barker	Hyacinthaceae	Namibia	Müller-Doblies H059	AJ232505	AJ232628	this paper
Lachenalia pallida Aiton	Hyacinthaceae	cult. B. G. Vienna	Pfosser H021	AJ232507	AJ232630	this paper
Ledebouria cordifolia (Baker) Stedje & Thulin	Hyacinthaceae	Malawi: Nyika plateau, Kongolo hills	Nordal & Stedje 2409	Z99143	Z99144	Stedje, 1998
Ledebouria revoluta (L. f.) Jessop	Hyacinthaceae	Zimbabwe: 8 km SW of Selous	Nordal 2082	Z99146	Z99147	Stedje, 1998
Ledebouria socialis (Baker) Jessop	Hyacinthaceae	cult. B. G. Vienna	Pfosser H014	AJ232501	AJ232624	this paper
Ledebouria somaliensis (Baker) Stedje & Thulin	Hyacinthaceae	Ethiopia: Bale region, 3.6 km S of Ginir	Nordal 2296	Z99150	Z99151	Stedje, 1998
Ledebouria urceolata Stedje in Stedje & Thulin	Hyacinthaceae	Ethiopia: Bale region, 8.4 km W of Goro	Nordal 2297	Z99148	Z99149	Stedje, 1998

Appendix 1. Continued.

Species	Family	Locality	Voucher°	EMBL acc. no. Intron	EMBL acc. no. IGS	Literature citation§
Loncomelos brachystylus (ZAHAR.) SPETA	Hyacinthaceae	Greece: Rhodos, Attaviros from Embona	Speta H008	AJ232487	AJ232610	this paper
Melomphis arabica (L.) RAF.	Hyacinthaceae	cult. B. G. Linz	Speta H004	AJ232482	JA232605	this paper
Merwilla lazulina (WILD) SPETA	Hyacinthaceae	Zimbabwe: 40 km NE of Rusape on road to Nyanga	Nordal 2491	Z99155	Z99156	Stedje, 1998
Merwilla sp. 1	Hyacinthaceae	cult. B. G. Vienna	Puff H219	AJ232499	AJ232622	this paper
Merwilla sp. 2	Hyacinthaceae	cult. B. G. Vienna	Puff H218	AJ232498	AJ232621	this paper
Muscari botryoides (L.) MILL.	Hyacinthaceae	Austria: Hasenufer near Pucking	Kleesadl H011	AJ232545	AJ232668	this paper
Muscari comosum (L.) MILL.	Hyacinthaceae	Italia: Monte	Neuner H056	AJ232546	AJ232669	this paper
Muscari macrocarpum SWEET	Hyacinthaceae	Turkey: Konya, 3 km outside of Göktepe, 1400 m	Fritsch H212	AJ232544	AJ232667	this paper
Nectaroscilla hyacinthoides (L.) PARL.	Hyacinthaceae	Portugal: Sierra da Amabina, close to Aleia de Irmaos (road to N. P.)	Scheiblreiter H016	AJ232542	AJ232665	this paper
Oncostema dimartinoi RAF.	Hyacinthaceae	Italia: Isola Marettimo, Egadi I., in colle Falcone, 400 m	Vezda H178	AJ232514	AJ232637	this paper
Oncostema peruviana (L.) SPETA	Hyacinthaceae	Portugal: Algarve, Vila do Bispo sul de Portugal (ex B. G. Lisboa 231/88)	Pfosser H198	AJ232516	AJ232639	this paper
Oncostema villosa (DESF.) RAF.	Hyacinthaceae	Tunisia: peninsula Bon, btw. Korbous and Douela, 90 m	Gruber H217	AJ232515	AJ232638	this paper
Ornithogalum amphibolum ZAHAR.	Hyacinthaceae	Romania: btw. Cochirlen and Rasova, 70 m	Speta H173	AJ232493	AJ232616	this paper
Ornithogalum angustifolium BOR. ($2n = 27$)	Hyacinthaceae	Poland: Krakow, Batowice (ex B. G. Amsterdam)	Raamsdonk H020	AJ232497	AJ232620	this paper
Ornithogalum fimbriatum WILLD.	Hyacinthaceae	Romania: btw. Babadag and Enisala	Speta H139	AJ232491	AJ232614	this paper
Ornithogalum gussonei TEN. acc. 1	Hyacinthaceae	Greece: Rhodos, Prof. Ilias, 770 m	Speta H164	AJ232489	AJ232612	this paper
Ornithogalum gussonei TEN. acc. 2	Hyacinthaceae	Greece: NW Peloponnes, pine forest near Araxos	Speta H166	AJ232488	AJ232611	this paper
Ornithogalum montanum TEN	Hyacinthaceae	Greece: Lafkas, Soulaki from Ajos Ilias, 600–1150 m	Speta H007	AJ232490	AJ232613	this paper
Ornithogalum pannonicum VILL.	Hyacinthaceae	Austria: Mödling, 200 m	Brandstätter H006	AJ232496	AJ232619	this paper

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Species	Family	Locality	Voucher°	EMBL acc. no. Intron	EMBL acc. no. IGS	Literature citation§
Ornithogalum pascheanum Speta	Hyacinthaceae	Turkey: Bolu, Abantsee, 1400–1600 m	Vasak H022	AJ232492	AJ232615	this paper
Ornithogalum umbellatum L. *aggr.* (2*n* = 45)	Hyacinthaceae	Slovakia: Bratislava, Kalinkovo, 80 m	Vasak H005	AJ232495	AJ232618	this paper
Ornithogalum wiedemannii Boiss.	Hyacinthaceae	Turkey: Yedi-Göller area, 30 km N of Bolu, 1850 m	Pasche H018	AJ232494	AJ232617	this paper
Othocallis sp.	Hyacinthaceae	Turkey: Nigde, Kamirkazik Dag, 1600 m	Pasche H179	AJ232533	AJ232656	this paper
Oziroe biflora (Ruiz & Pavon) Speta	Hyacinthaceae	Chile	MWC 793 (K)	AJ232453	AJ232576	this paper
Oziroe sp.	Hyacinthaceae	Chile: Prov. Copiapo, 1 km on Panamericana N of turnoff to Bahia Inglesa and Caldera, 100–160 m	Eggli & Leuenberger H362	AJ232452	AJ232575	this paper
Pfosseria bithynica (Boiss.) Speta	Hyacinthaceae	Bulgaria: Burgas, Aitoska Planina, Gorica, 300–400 m	Vasak H232	AJ232540	AJ232663	this paper
Polyxena calcicola U. & D. Müller-Doblies	Hyacinthaceae	South Africa	Müller-Doblies H216	AJ232506	AJ232629	this paper
Prospero elisae Speta acc. 1	Hyacinthaceae	Greece: Lefkas, Soulaki from Ajos Ilias, 1150 m	Speta H068	AJ232530	AJ232653	this paper
Prospero elisae Speta acc. 2	Hyacinthaceae	Greece: Parnass 1100 m	Ehrendorfer H155	AJ232531	AJ232654	this paper
Prospero haritonidae Speta	Hyacinthaceae	Greece: near Mikrothivae	Speta H027	AJ232528	AJ232651	this paper
Prospero obtusifolium (Poiret) Speta	Hyacinthaceae	Morocco: Beni-el Quidane, 1300 m	HC H053	AJ232529	AJ232652	this paper
Pseudogaltonia clavata (Masters) Phillips	Hyacinthaceae	cult. B. G. Vienna	Speta H220	AJ232475	AJ232598	this paper
Puschkinia scilloides Adams	Hyacinthaceae	cult. B. G. Linz	Speta H224	AJ232532	AJ232655	this paper
Rhadamanthus sp. 1	Hyacinthaceae	Madagascar: Antsalova region	Rafamantanantsoa H409	AJ232457	AJ232580	this paper
Rhadamanthus sp. 2	Hyacinthaceae	Madagascar: Antsalova region	Rafamantanantsoa H410	AJ232458	AJ232581	this paper
Schizocarphus nervosus (Burch.) Merwe	Hyacinthaceae	Zimbabwe: Chimanimani Mts.	Stedje 94/15	Z99157	Z99158	Stedje, 1998
Schnarfia messeniaca (Boiss.) Speta	Hyacinthaceae	Greece: 3 km NW of Kalamata, Mons Velalidis, 200 m	Vasak H177	AJ232543	AJ232666	this paper
Scilla albescens Speta	Hyacinthaceae	Greece: Crete, Psiloritis, Ida cave	Speta H237	AJ232553	AJ232676	this paper
Scilla cf. bulgarica * Speta	Hyacinthaceae	Romania: SE of Greci	Speta H158	AJ232555	AJ232678	this paper

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Species	Family	Locality	Voucher°	EMBL acc. no. Intron	EMBL acc. no. IGS	Literature citation§
Scilla cydonia Speta acc. 1	Hyacinthaceae	Greece: Crete, btw. Nea Roumata and Papadiana	Jahn et al. H215	AJ232549	AJ232672	this paper
Scilla cydonia Speta acc. 2	Hyacinthaceae	Greece: Karpathos: Kali Limni, ca. 1200 m	Speta H489	AJ232550	AJ232673	this paper
Scilla nana (J.A. & J.H. Schultes) Speta	Hyacinthaceae	Greece: Crete, Levka Ori, E. Kallergi, 1600 m	Speta H238	AJ232552	AJ232675	this paper
Scilla siehei (Stapf) Speta cv. "Pink Giant"	Hyacinthaceae	cult. LI	Speta H010	AJ232551	AJ232674	this paper
Scilla spetana Kereszty	Hyacinthaceae	Austria: Kreuttal near Unterolberndorf	Speta H227	AJ232556	AJ232679	this paper
Scilla subnivalis (Halacsy) Speta	Hyacinthaceae	Greece: Kephalonia, Änos, 1080–1630 m	Speta H240	AJ232554	AJ232677	this paper
Stellarioides ecklonii (Fisch. & Mey.) Speta	Hyacinthaceae	cult. B. G. Graz	Speta H289	AJ232470	AJ232593	this paper
Stellarioides etesiogaripensis (U. & D. Müller-Doblies) Speta	Hyacinthaceae	Namibia: farm Klein-Kubub, 1.5 km W of Rosh Pinah road	Müller-Doblies H138	AJ232469	AJ232592	this paper
Stellarioides longebracteata (Jacq.) Speta	Hyacinthaceae	cult. B. G. Vienna	Pfosser H407	AJ232471	AJ232594	this paper
Stellarioides tenuifolia (F. Delaroche) Speta	Hyacinthaceae	Uganda: Toro Distr., Queen Elizabeth Natl. Park, Mweya Peninsula	A. Bjornstad 548	Z99152	Z99153	Stedje, 1998
Thuranthos indicum (Roxb.) Speta sensu lato	Hyacinthaceae	Senegal: Région de Thiès, 2 km N of Mbour, near Sali Portugal	Ebert & Bangoura H202	AJ232455	AJ232578	this paper
Tractema lilio-hyacinthus (L.) Speta	Hyacinthaceae	Spain: Picos de Europa, at about 1450 m alt.	Hoog & Hoog H298	AJ232511	AJ232634	this paper
Tractema monophyllos (Link) Speta acc. 1	Hyacinthaceae	Spain: Cáceres, Salorino	Raus H049	AJ232513	AJ232636	this paper
Tractema monophyllos (Link) Speta acc. 2	Hyacinthaceae	Spain: Andalucia, Sierra de Cazorla 1200 m	W. & S. Till H306	AJ232512	AJ232635	this paper
Urginavia altissima (L. f.) Speta	Hyacinthaceae	Zimbabwe: 40 km NE of Gokwe	Nordal 2302	Z99133	Z99134	Stedje, 1998
Urginavia micrantha * (A. Rich.) Speta	Hyacinthaceae	Ethiopia: W-Schoa, Guder	Hildebrandt H439	AJ232460	AJ232583	this paper

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Species	Family	Locality	Voucher°	EMBL acc. no.		Literature citation§
				Intron	IGS	
Urginavia sp.	Hyacinthaceae	Guinea: Région de Moyenne Guinée, NW of Timbo	Ebert & Bangoura H357	AJ232461	AJ232584	this paper
Urginea sensu lato	Hyacinthaceae	Guinea: Moyenne Guinée, N of Pita, road to Hafia	Ebert & Bangoura H405	AJ232459	AJ232582	this paper
Urginea undulata (Desf.) Steinh.	Hyacinthaceae	Tunisia: Hammamet	Teppner H454	AJ232463	AJ232586	this paper
Veltheimia bracteata Harv. ex Baker	Hyacinthaceae	cult. LI	Speta H060	AJ232503	AJ232626	this paper
Whiteheadia etesionamibensis * Müller-Doblies	Hyacinthaceae	Namibia: Namuskluft	Lavranos & Pehlemann H444	AJ232504	AJ232627	this paper
Zagrosia persica * (Hausskn.) Speta acc. 1	Hyacinthaceae	Turkey: btw. Siirt and Bitlis	Leep H440	AJ232537	AJ232660	this paper
Zagrosia persica (Hausskn.) Speta acc. 2	Hyacinthaceae	Turkey: Beyez Dag, 1400 m	Stevens H500	AJ232538	AJ232661	this paper
Zahariadia saundersiae (Baker) Speta	Hyacinthaceae	South Africa (ex B. G. Berlin)	Speta H024	AJ232480	AJ232603	this paper
Arthropodium milleflorum (DC.) J.F. MacBr.	Lomandraceae	Australia: N.S.W., Barrington TOPS N. P., 1100–1400 m	Speta H418	AJ232436	AJ232559	this paper
Arthropodium sp.	Lomandraceae	Australia: N.S.W., Chichester State Forest, Jerusalem Creek, 380–490 m	Speta H423	AJ232435	AJ232558	this paper
Eustrephus latifolius R. Br.	Lomandraceae	Australia: NSW	Chase 193 (NCU)	AF117004	AF117032	Meerow et al., 1999
Thysanotus virgatus Brittan	Lomandraceae	Australia: NSW, Marley Track, Royal N. P.	Speta H413	AJ232437	AJ232560	this paper
Calibanus hookeri Trel.	Nolinaceae	Mexico: San Luis Potosi: Mts. near Balneario de Lourdes	Schatzl AG01	AJ232434	AJ232557	this paper
Bessera elegans Schult. f.	Themidaceae	Mexico: Sinaloa	Chase 626 (K)	AF117015	AF117040	Meerow et al., 1999
Brodiaea jolonensis Eastw.	Themidaceae	USA: California	Chase 1831 (K)	AF 117017	AF117046	Meerow et al., 1999
Dichelostemma pulchellum A. Heller	Themidaceae	cult. ex U of California, Irvine, Arboretum	—	—	—	Chase, unpublished
Milla magnifica H.E. Moore jr.	Themidaceae	—	Meerow 2309 (FLAS)	AF117011	AF117041	Meerow et al., 1999